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Growth of Enveloped RNA Viruses in a Line of Chinese Hamster Ovary Cells with Deficient *N*-Acetylglucosaminyltransferase Activity

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Sindbis and vesicular stomatitis viruses were grown in a line (termed 15B) of Chinese hamster ovary (CHO) cells that is deficient in a specific UDP-*N*-acetylglucosamine:glycoprotein *N*-acetylglucosaminyltransferase. Both viruses replicated normally in the cell line, but the glycoproteins of the released virus migrated faster on sodium dodecyl sulfate-polyacrylamide gels than did glycoproteins of virus grown in parent CHO cells. Digestion of the viral glycoproteins with Pronase followed by gel filtration demonstrated that the glycopeptides of Sindbis-15B virus were much smaller than the glycopeptides of Sindbis-CHO virus. In addition, Sindbis-15B viral glycopeptides but not Sindbis-CHO viral glycopeptides contained terminal α-mannose residues as shown by their susceptibility to α-mannosidase digestion. These findings demonstrate that the oligosaccharide units of the glycoproteins of vesicular stomatitis and Sindbis viruses are altered when the viruses are grown in 15B cells. We conclude that the *N*-acetylglucosaminyltransferase that is missing in 15B cells normally participates in the biosynthesis of the oligosaccharide units of the viral glycoproteins, and in the absence of this enzyme incomplete oligosaccharide chains are produced. Viruses released from 15B cells appear to retain full infectivity; Sindbis-15B virus, however, showed a significant decrease in hemagglutination titer compared with that of Sindbis-CHO virus.

Enveloped RNA viruses, such as Sindbis and vesicular stomatitis virus (VSV), contain glycoproteins on their surfaces. Recent data from several laboratories indicate that the carbohydrate moieties of these glycoproteins may be involved in the replication and infectivity of these viruses. Thus, the addition to virus-infected cells of either 2-deoxy-D-glucose or D-glucosamine, compounds that affect glycosylation of glycoproteins without impairing viral protein synthesis, inhibits the formation of infectious virus (4, 8, 9, 11, 20). Even more striking is the finding that decreasing the sialic acid content of VSV either by neuraminidase treatment (18) or by growth of the virus in mosquito cells (19) results in a marked loss in infectivity.

To pursue studies on the role of carbohydrate moieties of viral glycoproteins, it would be useful to be able to modify these molecules in a defined manner. Recently we isolated a clone (termed 15B) of Chinese hamster ovary (CHO) cells that have a deficiency of a specific UDP-*N*-acetylglucosamine:glycoprotein *N*-acetylglucosaminyltransferase activity (6). This enzyme deficiency results in the synthesis of membrane glycoproteins that have decreased amounts of *N*-acetylglucosamine, galactose, and sialic acid. We have grown Sindbis virus and VSV in this variant cell line to determine whether the host cell enzyme deficiency affects the synthesis of the carbohydrate units of the viral glycoproteins and, if so, whether the properties of the virus obtained from the variant cell are altered. The results of these experiments form the basis for this report.

MATERIALS AND METHODS

Materials. [^{3}H]glucosamine (56 mCi/mm) and [^{35}S]methionine (280 Ci/mm) were purchased from New England Nuclear Corp., Boston, Mass. Alpha-MEM was obtained from Flow Laboratories, Rockville, Md. Fetal calf serum, penicillin, and streptomycin were purchased from Grand Island Biological Co., Grand Island, N.Y. Pronase was purchased from Calbiochem, Los Angeles, Calif. Emulphogene, BC

720, an alkoxypoly(ethyleneoxy)ethanol, was obtained from GAF Corp., New York, N.Y. Purified α-mannosidase was prepared from jack bean meal as described previously (12). *Ricinus communis* agglutinin (RCA I) was prepared from castor beans as described previously (1). Sepharose 4B was obtained from Pharmacia Fine Chemicals, Inc., Piscataway, N.J.

Cells. The enzyme-deficient 15B line of CHO cells was selected with ricin, a highly toxic galactose-binding lectin, as described previously (7). Both parent and variant cells were grown as monolayers in alpha-MEM supplemented with 10% fetal calf serum, 50 U of penicillin per ml, and 50 μg of streptomycin per ml.

Growth of viruses. Nearly confluent monolayers of cells growing on 60-mm tissue culture petri dishes or 250-ml T flasks were infected with Sindbis virus at a multiplicity of infection of 200 to 400 PFU/cell. The virus was allowed to adsorb to the cells for 1 h in a volume of 0.5 ml for the petri dishes and 2.0 ml for the large T flasks, followed by removal of the unadsorbed inoculum. Then 4 and 6 ml of fresh medium were added, respectively, to the two types of growth vessels. Virus was harvested 12 to 16 h after infection.

For VSV growth, monolayers of cells in 250-ml T flasks were infected at a multiplicity of infection of 10 PFU/cell in 2.0 ml of medium for 1 h. After removal of the infecting medium, 7 ml of fresh medium was added. Virus was harvested 6 to 9 h later.

Radioactive labeling of viruses. To label Sindbis virus with [^{35}S]methionine, the medium was removed 5 h after infection and replaced with the same volume of MEM lacking amino acids and containing 10% fetal calf serum plus 10 μCi of [^{35}S]methionine per ml. When [^{3}H]glucosamine was added, it was added 1 to 3 h postinfection at 25 μCi/ml. For the double-label experiments, the [^{3}H]glucosamine was added to MEM lacking amino acids and 10% fetal calf serum 2 to 3 h postinfection; the [^{35}S]methionine was added 2 to 3 h thereafter. To label the VSV, the medium was replaced with amino acid-deficient MEM containing 10% fetal calf serum and [^{35}S]methionine 4 h postinfection. In all cases, the virus was harvested at the usual time postinfection, as described for unlabeled viruses.

Preliminary experiments with [^{3}H]glucosamine-labeled virus from both cell types revealed that virtually all of the label was distributed among *N*-acetylglucosamine and sialic acid residues, demonstrating that any radioactive label derived from [^{3}H]glucosamine reflects carbohydrate content.

Virus purification. Sindbis virus was purified by centrifugation to equilibrium in a sucrose gradient as described previously (23). VSV was purified by the method of Schloemer and Wagner (18).

Determination of PFU. Harvested media were diluted and applied onto monolayers of chicken embryo fibroblasts, baby hamster kidney cells, or mouse L cells as indicated for each experiment, either before or after storage at −70 C, as described previously (16). Although freezing did result in reduction of plaque-forming ability, the relative PFUs of the virus per milliliter produced by the different cell lines did not alter significantly.

Hemagglutination assay. Agglutination of gander erythrocytes by Sindbis virus was performed as described previously (2).

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. Samples of gradient-purified, [^{35}S]methionine-labeled VSV and Sindbis virus were reduced and alkylated prior to electrophoresis on discontinuous slab or cylindrical polyacrylamide gels as described elsewhere (17). The slab gels were subjected to autoradiography, and the cylindrical gels were sliced for radioactivity counting in scintillation fluid.

Sugar analysis of [^{3}H]glucosamine-labeled virus. Gradient-purified virus was dialyzed extensively against 0.45% NaCl and analyzed for the distribution of incorporated radioactivity as described previously (6).

RCA-I affinity chromatography of viral glycoproteins. RCA-I was coupled to Sepharose 2B by a modification (1) of the cyanogen bromide procedure of Cuatrecasas (3). The lectin-Sepharose was poured into columns (0.75 by 5 cm) and equilibrated with 0.5% Emulphogene in water. Gradient-purified, [^{35}S]methionine-labeled virus was dialyzed against 0.45% saline. Then Emulphogene and $NaHCO_3$ were added to a final concentration of 0.5% and 0.01 M, respectively, to solubilize the viral proteins. After 1 h at 4 C, the viral solution was passed through the RCA I-Sepharose column at room temperature. The column was washed with 20 ml of 0.5% Emulphogene followed by 20 ml of 1 M NaCl in 0.5% Emulphogene. Finally, the column was eluted with 0.1 M lactose in 0.5% Emulphogene in 0.9% NaCl-0.01 M $NaHCO_3$. The unadsorbed and the lactose-eluted fractions were dialyzed against 6 liters of water and concentrated to 1 ml. The fractions were extracted five times with 2 volumes of toluene to remove the bulk of the detergent and then prepared for SDS-polyacrylamide gel electrophoresis as described above.

Purification of viral glycopeptides. Gradient-purified virus labeled with [^{3}H]glucosamine was incubated with 1% Pronase (wt/vol) in 0.1 M PO_4 (pH 7.8) containing 0.1% SDS for 4 days at 37 C under a toluene atmosphere. Fresh Pronase (0.1%) was added daily. The incubation mixture was then heated at 100 C for 3 min and centrifuged to remove the particulate material. The supernatant solution was applied to a Sephadex G-50 column (1.6 by 50 cm) and was eluted with 0.9% NaCl. The fractions containing the Sindbis-CHO glycopeptides were pooled, dialyzed against water, and applied to a DEAE-cellulose column (1 by 2.3 cm) equilibrated with 1 mM PO_4, pH 7.5. One species of glycopeptide material eluted in the starting buffer, whereas a second glycopeptide fraction was eluted with 15 ml of 10 mM PO_4, pH 7.5. These two fractions were then dialyzed and reapplied to the Sephadex G-50 column. The resulting fractions were again pooled, dialyzed, and concentrated. The pooled Sindbis-15B fractions from the first Sephadex G-50 column were similarly dialyzed and concentrated. All three pooled fractions were then subjected to enzyme digestion with purified jack bean α-mannosidase in 0.05 M citrate (pH 4.6) for 3 days and were then rerun on the Sephadex G-50 column.

Fractions were monitored by radioactivity counting.

Protein determination. Protein was determined by the method of Lowry et al. (14).

RESULTS

Mobility of viral glycoproteins on polyacrylamide gels. Our first test for the comparison of viruses produced by CHO or 15B cells was to analyze their proteins by electrophoresis on polyacrylamide gels. Viruses grown in the presence of [^{35}S]methionine to label the proteins were purified by centrifugation in a sucrose gradient. The reduced and alkylated viral proteins were then subjected to electrophoresis on polyacrylamide slab gels in the presence of SDS (Fig. 1). In the case of Sindbis virus, both

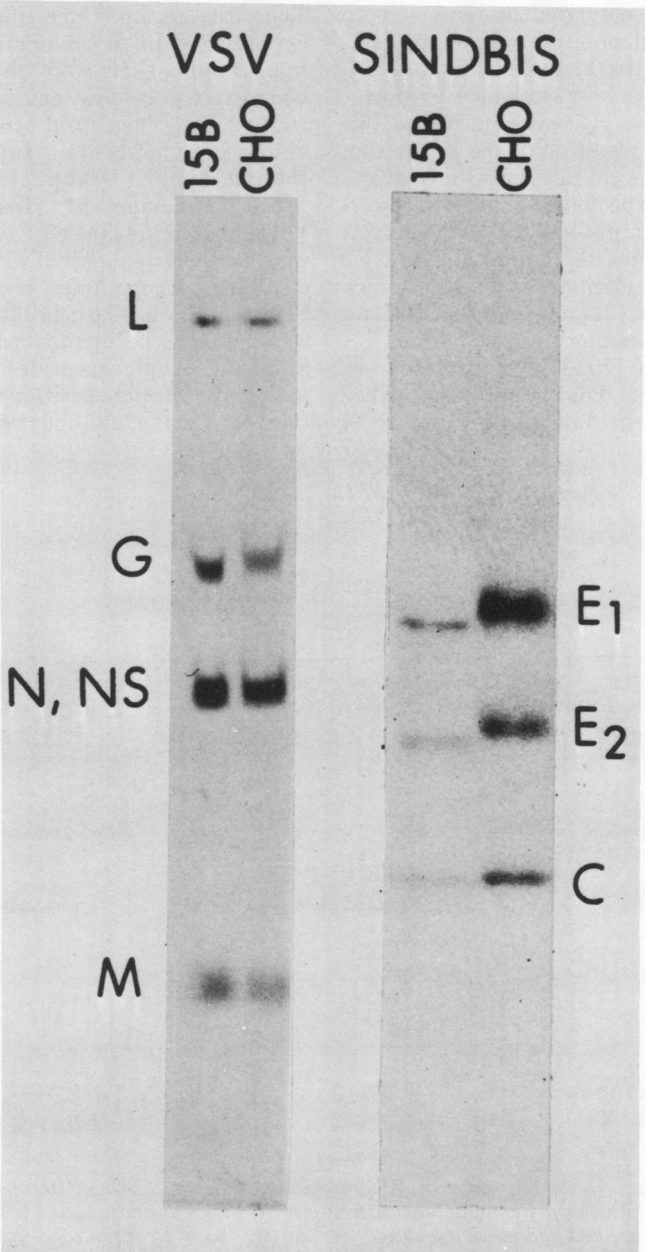

FIG. 1. *Autoradiograms of SDS-polyacrylamide gels of [^{35}S]methionine-labeled VSV and Sindbis virus grown in CHO and 15B cells. G denotes the single glycoprotein found in VSV, whereas E1 and E2 are the two glycoproteins of the Sindbis virus. L, N, NS, M, and C are non-glycosylated viral proteins.*

glycoproteins (E1 and E2) of the virus produced in 15B cells migrated more rapidly than their counterparts from virus grown in parent CHO cells. In contrast, the mobility of the non-glycosylated capsid protein (C) of both viruses was identical. Similarly, the VSV glycoprotein (G) of virus grown in 15B cells migrated faster than the viral glycoprotein of parent cells, whereas the non-glycosylated proteins of both viruses had identical mobilities. Given the demonstrated glycosylation defect in the 15B cell line, these results are best explained by postulating a block in the glycosylation of the 15B viral glycoproteins, resulting in the production of incomplete oligosaccharide units. Such glycoproteins would be expected to migrate as lower-molecular-weight forms on SDS-polyacrylamide gels (22). The finding that both E1 and E2 are altered in glycosylation was expected since Sefton and Keegstra (21) reported that the two glycoproteins have similar oligosaccharides.

Fractionation of viral glycoproteins on RCA I-Sepharose. The membrane glycoproteins of 15B cells fail to bind to RCA I-Sepharose affinity columns because of their altered glycosylation. In contrast, approximately 50% of the parent CHO membrane glycoproteins bind to the affinity column and can be eluted with the haptene sugar lactose (6). As shown in Fig. 2, the solubilized glycoproteins of Sindbis virus from the two cell types reflected this differential binding behavior. Thus, the glycoproteins from Sindbis-15B did not bind to RCA I-Sepharose, whereas those from Sindbis-CHO were almost quantitatively bound to the column and subsequently eluted with lactose. The capsid protein (C) from these virions does not contain any carbohydrate and was not retained by the column.

Gel filtration of viral glycopeptides. [^{3}H]glucosamine-labeled viral glycoproteins were extensively digested with Pronase, and the resultant glycopeptides were fractionated on Sephadex G-50. The Sindbis-15B glycopeptides were found to be significantly smaller than the Sindbis-CHO glycopeptides (Fig. 3A). By comparing the elution position of these glycopeptides with that of glycopeptides of known molec-

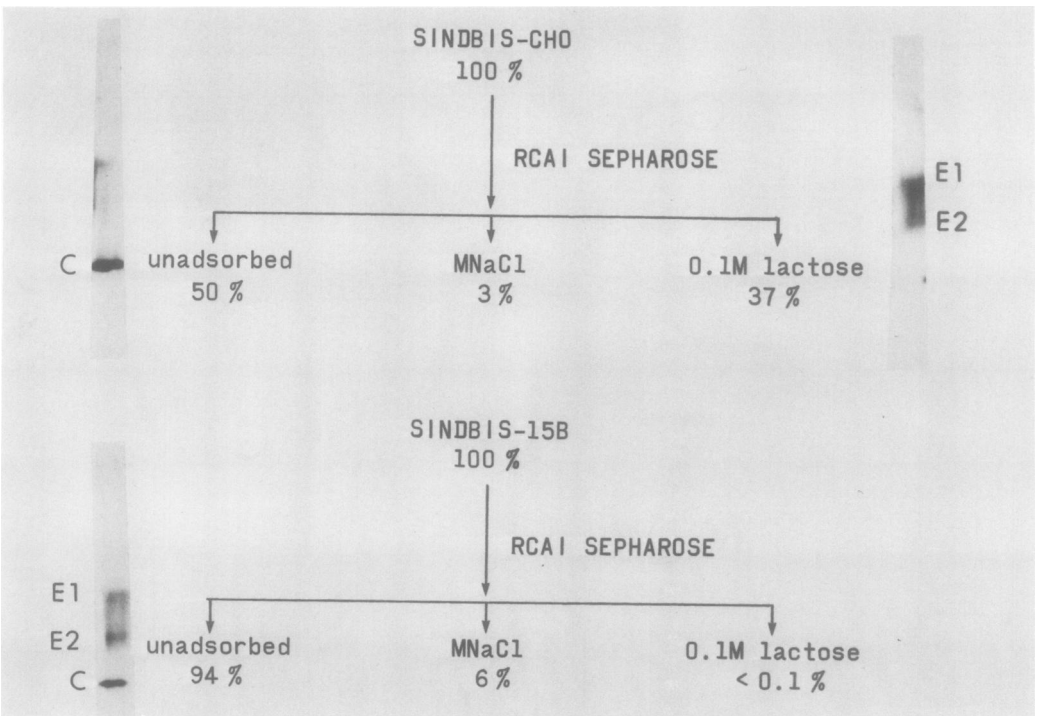

FIG. 2. *RCA-I affinity chromatography of [^{35}S]methionine-labeled Sindbis-CHO and -15B. The labeled virus was solubilized with the nonionic detergent Emulphogene (0.5%) and applied to the RCA-I Sepharose column. The percent applied radioactivity eluted sequentially with Emulphogene in buffer, 1 M NaCl in Emulphogene and 0.1 M lactose is shown. The Sindbis-CHO and -15B unadsorbed fractions and the lactose eluate of Sindbis-CHO were subjected to SDS-polyacrylamide electrophoresis. The autoradiograms of the gels are shown above, adjacent to their respective column fractions.*

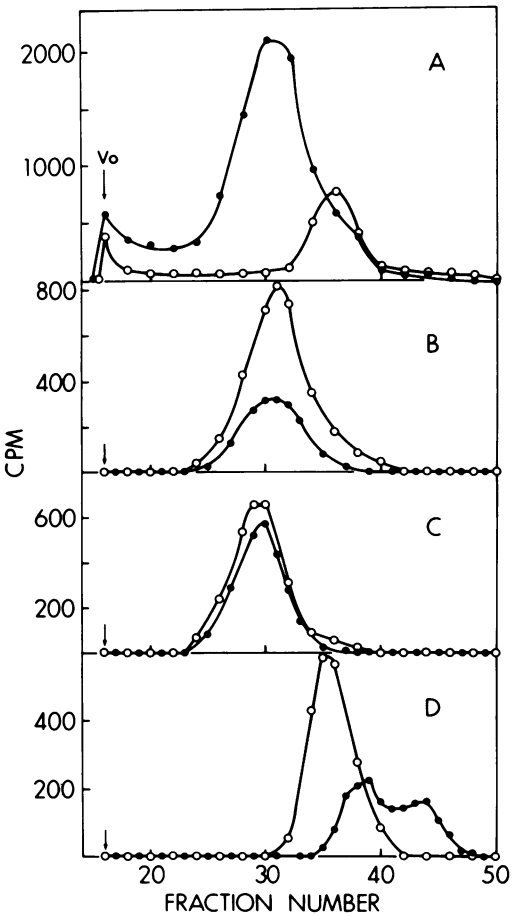

FIG. 3. *Sephadex G-50 chromatography of [^{3}H]glucosamine-labeled viral glycopeptides of Sindbis-CHO and -15B. (A) Labeled viruses were digested with Pronase and chromatographed in 0.9% NaCl on a calibrated Sephadex G-50 column. Symbols: ●, CHO; ○, 15B. The CHO peak was pooled, dialyzed against water, and applied to a DEAE-cellulose column in 1 mM phosphate buffer. (B) The Sindbis-CHO glycopeptide fraction not adsorbed by the DEAE-cellulose column was rechromatographed on the G-50 column. The elution pattern is shown (○). The material in this peak was pooled, treated with α-mannosidase, and then rechromatographed on the G-50 column (●). (C) That portion of the pooled Sindbis-CHO glycopeptide which was eluted from the DEAE-cellulose column with 10 mM PO_4 buffer was also rechromatographed on the G-50 column (○). This material was then treated with α-mannosidase and rechromatographed on the G-50 column (●). (D) The pooled Sindbis-15B glycopeptide material from (A) was treated with α-mannosidase and rerun on the G-50 column (●). The repeat elution pattern on the G-50 column of the pooled, untreated Sindbis-15B glycopeptide from (A) is shown (○).*

ular weight, we estimated that the Sindbis-15B glycopeptides had a molecular weight of about 1,360, whereas the Sindbis-CHO glycopeptides had a molecular weight of about 2,100. The Sindbis-CHO glycopeptides were further separated into two fractions by DEAE-cellulose chromatography. One of the glycopeptides had an estimated molecular weight of 2,040 (Fig. 3B), whereas the other had an estimated molecular weight of 2,200 (Fig. 3C).

If one assumes that the specific UDP-*N*-acetylglucosamine:glycoprotein *N*-acetylglucosaminyltransferase that is absent in 15B cells is required for the synthesis of complete oligosaccharide units of the viral glycoproteins, one would expect the small Sindbis-15B glycopeptides to contain oligosaccharide cores that terminate in α-mannose residues, since the acceptors for the missing *N*-acetylglucosaminyltransferase are oligosaccharides with terminal α-mannose residues (6). The Sindbis-CHO glycoproteins, on the other hand, should be completed oligosaccharide units which probably terminate in sugars other than mannose. To demonstrate this, the various glycopeptides were incubated with highly purified α-mannosidase and then again passed through the Sephadex G-50 column (Fig. 3B, C, and D). Whereas the Sindbis-CHO glycopeptides did not change their elution position, the Sindbis-15B glycopeptides now eluted as two lower-molecular-weight species, consistent with the enzymatic removal of one and two mannose residues.

Biological consequences of altered host and viral glycoproteins. Although our results clearly demonstrate that the glycoproteins of viruses produced by 15B cells are altered in their oligosaccharide units, we have been unable to detect any major effect of this alteration on infectivity. Growth curves of Sindbis virus in CHO and 15B cells did not show any notable differences in the rate of virus release from the two cell lines. The yields of PFUs for both Sindbis virus and VSV were essentially identical, and VSV-15B showed the same ability as VSV-CHO to plaque on several different cell monolayers (Table 1).

Schloemer and Wagner (18, 19) have reported that the PFU/particle ratio is greatly decreased when sialic acid is not present on the glycoproteins of VSV. To determine whether this ratio is affected in VSV obtained from 15B cells, we measured infectivity and protein for purified VSV from the two cell lines (Table 2). In this experiment, the particle determination was based both on the standard Lowry test (14) and

on the amount of [^{35}S]methionine incorporated into viral protein. The data show that the PFU/protein ratio for VSV-15B and VSV-CHO is essentially the same (Table 2).

We also compared the ratio of PFU to [^{35}S]methionine-labeled protein for Sindbis virus released from CHO and 15B cells and found no difference. However, we observed that the hemagglutinating activity of Sindbis-15B is significantly less than that of Sindbis-CHO (Table 3). In five different preparations of Sindbis virus from CHO and 15B cells, the difference in hemagglutination titer normalized to PFUs or counts per minute varied between 4- and 16-fold. The oligosaccharide structure responsible for the hemagglutinating activity of togaviruses such as Sindbis virus is not known, but removal of sialic acid does not affect this activity (10).

TABLE 1. *Virus yield from CHO and 15B cells*[a]

Virus source	PFU/cell		
	CEF	L	BHK
Sindbis-CHO	1.9×10^4	ND[b]	ND
-15B	2.1×10^4	ND	ND
VSV-CHO	0.9×10^8	0.8×10^8	1.4×10^8
-15B	1.7×10^8	2.6×10^8	2.1×10^8

[a] CHO and 15B monolayers were infected with Sindbis virus (previously grown in BHK cells) and VSV (previously grown in L cells). The harvested media were plaqued onto chicken embryo fibroblast (CEF), mouse L cell, and baby hamster kidney (BHK) cell monolayers. The results are expressed as PFUs per CHO or 15B cell.

[b] ND, Not determined.

DISCUSSION

Our experiments demonstrate that the oligosaccharide units of the glycoproteins of Sindbis and VSV viruses are altered when the viruses are grown in a host cell that is deficient in a particular *N*-acetylglucosaminyltransferase. The simplest explanation for these data is that the glycosyltransferase that is missing in 15B cells normally participates in the biosynthesis of the oligosaccharide units of the viral glycoproteins and, in the absence of this enzyme, incomplete oligosaccharide units are produced. To demonstrate this conclusively will require a more detailed analysis of the structure of the oligosaccharide units of both Sindbis-CHO and Sindbis-15B viral glycoproteins; such studies have been initiated.

Our preliminary studies indicate that glycoproteins from 15B cells or from virus produced by 15B cells are deficient in sialic acid. Schloemer and Wagner demonstrated that VSV in which the sialic acid is removed after treatment with neuraminidase (18) or is absent after the virus is grown in mosquito cells (19) has greatly reduced infectivity. Since VSV obtained from 15B cells appears fully infectious, it seems that those sialic acid residues critical for adsorption of the virus to cells must be retained in the virus produced by this particular variant cell line.

There are a number of implications of these experiments which deserve special comment. First, this work adds to the growing body of evidence that host cell glycosyltransferases are involved in the synthesis of the oligosaccharide units of viral glycoproteins. Several investiga-

TABLE 2. *Comparison of the PFU/protein ratio of VSV-CHO and VSV-15B*[a]

Virus	PFU/ml ($\times 10^{10}$)	mg of protein/ml	^{35}S counts/min per ml ($\times 10^8$)	PFU/mg of protein ($\times 10^{10}$)	PFU/counts per min ($\times 10^4$)
VSV-CHO	2.3	0.60	7.0	3.8	3.2
VSV-15B	3.5	0.85	11.7	4.1	3.0

[a] CHO and 15B cell monolayers were infected with VSV, and [^{35}S]methionine was added 4 h later. After purification, viruses were plaqued onto BHK monolayers, and protein was determined by the Lowry test and by radioactivity.

TABLE 3. *Comparison of the PFUs and hemagglutination of Sindbis-CHO and Sindbis-15B*[a]

Virus	PFU/ml ($\times 10^{10}$)	HA/ml	^{35}S counts/min per ml ($\times 10^8$)	PFU/counts per min ($\times 10^8$)	HA/^{35}S counts/min ($\times 10^{-8}$)
Sindbis-CHO	5.7	256	3.0	1.9	85
Sindbis-15B	3.6	32	1.8	2.0	18

[a] CHO and 15B cell monolayers were infected with Sindbis virus, and [^{35}S]methionine was added 5 h later. The gradient-purified viruses were plaqued onto chicken embryo fibroblast monolayers and tested for hemagglutination (HA) of gander erythrocytes at pH 5.8.

tors have shown previously that the size or the composition of the oligosaccharide units of the VSV glycoprotein varies depending on the cell type in which the virus is grown (5, 15). In addition, Moyer and Summers reported an alteration in the structure of the core region of the VSV G protein oligosaccharides when the virus is grown in polyoma-transformed baby hamster kidney (BHK) cells rather than in an untransformed cell line (15). The participation of host enzymes in viral glycoprotein biosynthesis is not an unexpected finding since the genome of the Sindbis virus and VSV does not contain enough information to code for the numerous glycosyltransferases required for the synthesis of complex oligosaccharides. Of more interest is the fact that the system described in this paper can be used to study the role of the oligosaccharide units of viral glycoproteins. Based on studies with 2-deoxyglucose, it has been suggested that the glycosylation of viral glycoproteins is essential for the replication of several enveloped RNA viruses (4, 8, 9, 11, 20). Our experiments demonstrate that the replication of Sindbis virus and VSV occurs normally in spite of incomplete glycosylation of the viral glycoproteins. These two findings are compatible since 2-deoxyglucose probably acts as a mannose analogue (9) and would interfere, therefore, with oligosaccharide biosynthesis at a point proximal to the block in 15B cells. A logical extension of our current work would be to prepare mutant cell lines that are unable to add mannose residues to the viral oligosaccharide chains and to study viral replication in such cell lines. The only alteration in viral function we have detected thus far in the Sindbis-15B virus is a decrease in hemagglutinating ability. However, if viruses could be prepared with other types of oligosaccharide abnormalities, the functional consequences might be more striking.

Another potential application of this system is to use the virus as a probe for determining the structure of the oligosaccharide units of membrane glycoproteins in cell lines that have subtle changes difficult to detect by routine techniques. The advantage of this approach is that the enveloped viruses have only one or two glycoproteins in contrast to the plasma membrane of the host cell, which contains numerous glycoproteins. Therefore, if the viral glycoproteins "pick-up" an oligosaccharide alteration that occurs in only a minority of the host cell glycoproteins, the probability of detecting such an alteration will be greatly enhanced. Moyers and Summers have shown that such an approach can be used to detect subtle changes in oligosaccharide structure induced by polyoma transformation of BHK cells (15).

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LITERATURE CITED

1. Adair, W. L., and S. Kornfeld. 1974. Isolation of the receptors for wheat germ agglutinin and the *Ricinus communis* lectins from human erythrocytes using affinity chromatography. J. Biol. Chem. **249**:4696-4704.
2. Clarke, D. H., and J. Casals. 1958. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. Am. J. Trop. Med. Hyg. **7**:561-573.
3. Cuatrecasas, P. 1970. Protein purification by affinity chromatography. J. Biol. Chem. **245**:3059-3065.
4. Duda, E., and M. Schlesinger. 1975. Alterations in Sindbis viral envelope proteins by treating BHK cells with glucosamine. J. Virol. **15**:416-419.
5. Etchison, J., and J. Holland. 1974. Carbohydrate composition of the membrane glycoprotein of vesicular stomatitis-virus grown in four mammalian-cell lines. Proc. Natl. Acad. Sci. U.S.A. **71**:4011-4014.
6. Gottlieb, C., J. Baenziger, and S. Kornfeld. 1975. Deficient uridine diphosphate-N-acetylglucosamine:glycoprotein N-acetylglucosaminyl-transferase activity in a clone of Chinese hamster ovary cells with altered surface glycoproteins. J. Biol. Chem. **250**:3303-3309.
7. Gottlieb, C., A. M. Skinner, and S. Kornfeld. 1974. Isolation of a clone of Chinese hamster ovary cells deficient in plant lectin-binding sites. Proc. Natl. Acad. Sci. U.S.A. **71**:1078-1082.
8. Kaluza, G., M. F. G. Schmidt, and C. Scholtissek. 1973. Effect of 2-deoxy-D-glucose on the multiplication of Semliki Forest virus and the reversal of the block by mannose. Virology **54**:179-189.
9. Kaluza, G., C. Scholtissek, and R. Rott. 1972. Inhibition of the multiplication of enveloped RNA-viruses by glucosamine and 2-deoxy-D-glucose. J. Gen. Virol. **14**: 251-259.
10. Kennedy, S. 1974. The effect of enzymes on structural and biological properties of Semliki Forest virus. J. Gen. Virol. **23**:129-143.
11. Klenk, H.-D., C. Scholtissek, and R. Rott. 1972. Inhibition of glycoprotein biosynthesis of influenza virus by D-glucosamine and 2-deoxy-D-glucose. Virology **49**:723-734.
12. Kornfeld, R., and S. Kornfeld. 1970. The structure of a phytohemagglutinin receptor site from human erythrocytes. J. Biol. Chem. **245**:2536-2545.
13. Lenard, J., and R. Compans. 1974. The membrane structure of lipid-containing viruses. Biochim. Biophys. Acta **344**:51-93.
14. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
15. Moyer, S. A., and D. F. Summers. 1974. Vesicular stomatitis virus envelope glycoprotein alterations induced by host cell transformation. Cell **70**:63-70.
16. Pfefferkorn, E. R., and H. S. Hunter. 1963. Purification

and partial chemical analysis of Sindbis virus. Virology **20**:443–445.
17. Schlesinger, S., and M. Schlesinger. 1972. Formation of Sindbis virus proteins: identification of a precursor for one of the envelope proteins. J. Virol. **10**:925–932.
18. Schloemer, R. H., and R. Wagner. 1974. Sialoglycoprotein of vesicular stomatitis virus: role of the neuraminic acid in infection. J. Virol. **14**:270–281.
19. Schloemer, R. H., and R. R. Wagner. 1975. Mosquito cells infected with vesicular stomatitis virus yield unsialylated virions of low infectivity. J. Virol. **15**:1029–1032.
20. Schwarz, R. T., and H.-D. Klenk. 1974. Inhibition of glycosylation of the influenza virus hemagglutinin. J. Virol. **14**:1023–1034.
21. Sefton, B., and K. Keegstra. 1974. Glycoproteins of Sindbis virus: preliminary characterization of the oligosaccharides. J. Virol. **14**:522–530.
22. Segrest, J. P., R. L. Jackson, E. P. Andrews, and V. T. Marchesi. 1971. Human erythrocyte membrane glycoprotein: a re-evaluation of the molecular weight as determined by SDS polyacrylamide gel electrophoresis. Biochem. Biophys. Res. Commun. **44**:390–395.
23. Weiss, B., and S. Schlesinger. 1973. Defective interfering passages of Sindbis virus: chemical composition, biological activity, and mode of interference. J. Virol. **12**:862–871.